IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Floriano, et al.	Art Unit	: 1631
Serial No.	: 10/010,725	Examiner	: Pablo S. Whaley
Filed	: November 30, 2001	Conf. No.	: 4307
Title	: METHODS AND APPARATUS FOR PREDICTING LIGAND BINDING INTERACTIONS		

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DECLARATION OF WILLIAM A. GODDARD, III UNDER 37 C.F.R. § 1.132

I, William A. Goddard, III, declare as follows:

I. I am the Charles and Mary Ferkel Professor of Chemistry, Materials Science, and Applied Physics, and the Director of the Materials and Process Simulation Center at the California Institute of Technology, and I am an inventor of the above-identified patent application.

II. The claimed invention, e.g., claims 1, 31, and 48, relates to methods, computer program products, and systems for identifying one or more ligand conformations that bind to a protein.

III. On November 30, 2001, the filing date of the subject application, computational methods for predicting ligand binding sites in proteins, modeling protein-ligand interactions and drug design could be described in two general categories: (1) thorough, but computationally expensive methods that could predict configurations for certain protein-ligand combinations, but were impractical or intractable in application to the large numbers of ligands typically of interest in drug design; (2) various methods which made significant compromises in thoroughness and accuracy in order to achieve computational practicality and tractability. Consequently, on the filing date of the subject application, the trade-offs between practicality and accuracy were well-known.

The complexity of the trade-off problem is compounded in any practical approach to drug design because of the number of possible configurations for a particular ligand at each possible binding site in a particular protein, the number of possible ligands that a drug designer may seek to evaluate, and, for proteins for which the binding site was not known, the large number of possible binding sites on such a protein for such ligands. For example, on the filing date of the subject application, most, if not all practical computational methods (category (2)) for predicting ligand binding sites in proteins, modeling protein-ligand interactions and drug design were docking methods which used one docking procedure for each configuration, using the same or about the same level of computation for every configuration examined. In a typical example of such methods, an algorithm is applied to each of a large number of configurations and binding structures (generated, e.g., by Monte Carlo methods) and the best calculated configuration is selected. Thus, under such methods, computational requirements increase with both the number of configurations to be calculated and the level of accuracy for each calculation. However, such methods were largely unsuccessful in identifying the best configurations for any moderately complex ligand-protein systems of interest because of the extremely large number of possible configurations to be calculated and the level of accuracy required for each such calculation leads to computational requirements exceeding even the fastest available supercomputers of the time. For example, Zou *et al. J. Am. Chem. Soc.*, 1999, Vol. 121, p.8033-8043, are highly skeptical of combining, e.g., solvent interactions and molecular dynamics, and conclude that such a combination would be impractical:

> Accounting for the effect of solvent on the strength of molecular interactions has been a longstanding problem for molecular calculations in general and for structure-based drug design in particular. (abstract).

> The most obvious method to overcome these problems is to treat solvent molecules explicitly in molecular dynamics or Monte Carlo simulations of binding ...However, these approaches are currently impractical for screening large numbers of molecules. (p. 8043, last paragraph, through page 8044, line 3).

Moreover, such problems were not limited to calculating solvent interactions specifically, but generally applied to combined methods in calculations for many ligand-protein systems of pharmaceutical interest, as the number of configurations to be calculated and the level of accuracy needed led to impractical or intractable computational problems.

Also, on the filing date of the subject application, category (1) methods (e.g., full molecular dynamics simulation of a membrane protein and a selected ligand, using infinite membrane and solvent effects) had significant limitations, even on a particular protein and particular ligand. For example, on the filing date of the subject application, no computation had ever predicted an accurate drug ligand-protein configuration for a new drug ligand-protein combination in advance of experimental studies to locate the binding region of the protein.

IV. By contrast, the references in Section VI below show that exemplary implementations of the claimed invention ("HierDock") predict surprisingly accurate binding sites and configurations for a number of proteins and ligands in a practical, tractable manner. In each case, an example of the claimed invention was employed to sample the entire protein to locate the binding site and to refine the protein-ligand configurations at the binding site to lead to accurate configurations. These predictions have been validated by full molecular dynamics simulation using infinite membrane and solvent effects, and in some cases by actual experimental mutation studies.

For example, references 7, 8, and 9 show that protein-ligand configurations predicted by an exemplary implementation of the claimed invention are surprisingly stable and accurate when validated with extensive molecular dynamics simulations using infinite membrane and solvent effects.

Ligand-protein configurations predicted by exemplary implementations of the claimed invention (references 6, 8, and 9) were employed to design subsequent mutation experiments, which validated the surprising accuracy of the predicted configurations.

In particular, the work reported in reference 9 permitted our collaborator, Sanofi-Aventis, to design a new drug for allergic inflammation, which is now in advanced trials. Reference 9 shows that the predicted ligand structures give details of the relative binding energies for surprisingly closely related ligands, even for cases differing by only a factor of two in binding constant. For example, reference 9 shows that an exemplary implementation of the claimed invention surprisingly and correctly predicted the best ligand, which was subsequently shown to have a binding constant 1000 times better (0.8 nM versus 800 nM) than the lead compound identified by traditional pharmaceutical methods. This demonstrates that the subject invention provides practical, tractable prediction protein-ligand configurations at a level of accuracy sufficient to provide surprising and unexpected improvements in drug design in comparison to the traditional methods used to identify the initial candidate. In particular, no other computational approach has ever correctly predicted protein-ligand structures for a membrane protein in advance of experimental studies to locate the binding region of the protein, let alone resulting in a potentially important new drug.

Finally, another surprising result of the claimed invention is that in combination with the above accuracy, it is practical and tractable enough to be employed on a laptop or workstation computer without requiring a supercomputer. The combination of claimed steps permits efficient allocation of computational resources so that, for example, large numbers of ligands or ligand conformations can be addressed at the step of "applying a coarse-grained docking algorithm to identify a plurality of binding conformations for the one or more ligands in the binding region," while still permitting the step of "calculating a binding energy for each conformation of the preferred set of conformations" to be performed at a high level.

V. The references below in Section VI include pharmaceutically relevant, financially successful embodiments of the claimed invention, resulting in over $2.5M received from major pharmaceutical companies to date. This success is because the claimed invention addresses a long felt need in the pharmaceutical industry for a practical method that can predict new protein-ligand configurations, allowing structure based drug design methods to effectively research important targets such as G-protein coupled receptors. Further, the success permitted by the claimed invention could not be performed by pharmaceutical companies in house using available programs . Consequently, even though these pharmaceutical companies typically do not want their research directions to be known to the outside world, they fund this commercially relevant work at the Materials and Process Simulation Center at the California Institute of Technology, as evidenced by corresponding support statements in the Section VI references.

Regarding financial success, for example: Boehringer-Ingelheim provided funds of $750K for projects over a three-year period; Berlex (previously part of Schering AG, now Bayer AG) provided $300K in funds over 2 years for the work described in reference 6; Pfizer has provided $450K over a two year period; Alloyzne has provided $270K; and the work reported in reference 9 was supported by Sanofi-Aventis at $750K over a three-year period.

Regarding pharmaceutical success relevant to the long-felt need in the pharmaceutical industry, the work reported in reference 9 for Sanofi-Aventis is the first time that theory has led to a new drug for a G-protein coupled receptor, which drug has entered advanced trials for allergic inflammation. No other computational approach has ever correctly predicted protein-ligand structures for a membrane protein in advance of experimental studies to locate the binding region of the protein, let alone resulting in a potentially important new drug.

VI. References

1. **The predicted 3D structure of the human D2 dopamine receptor and the binding site and binding affinities for agonists and antagonists**; Yashar M, Kalani S, Vaidehi N, Hall SE, Trabanino RJ, Freddolino PL, Kalani MA, Floriano WB, Kam VWT, Goddard WA; Proc. Nat. Acad. Sci., **101** (11): 3815-3820 (**2004**)
2. **Predicted 3D structure for the human beta 2 adrenergic receptor and its binding site for agonists and antagonists**; Freddolino PL, Kalani MYS, Vaidehi N, Floriano WB, Hall SE, Trabanino RJ, Kam VWT, Goddard WA; Proc. Nat. Acad. Sci., **101** (9): 2736-2741 (**2004**)
3. **Test of the Binding Threshold Hypothesis for olfactory receptors: Explanation of the differential binding of ketones to the mouse and human orthologs of olfactory receptor 912-93**; Hummel P, Vaidehi N, Floriano WB, Hall SE, Goddard WA; Protein Science **14** (3): 703-710 (**2005**)
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5. **The Predicted 3D Structures of the Human M1 Muscarinic Acetylcholine Receptor with Agonist or Antagonist Bound**; Joyce Yao-chun Peng, Nagarajan Vaidehi, Spencer E. Hall, William A. Goddard III; ChemMedChem, **1** (8): 878-890 (**2006**)
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7. **Dynamic behavior of fully solvated beta 2-adrenergic receptor, embedded in the membrane with bound agonist or antagonist;** Spijker, P; Vaidehi, N; Freddolino, PL; Hilbers, PAJ; Goddard, WA; P. Natl. Acad. Sci., **103** (13): 4882-4887 (**2006**)
8. **Prediction of the 3D structure for FMRF-amide Neuorpeptides Bound to the Mouse MrgC11 GPCR and Experimental Validation**; Jiyoung Heo, Sang-Kyou Han, Nagarajan Vaidehi, John Wendel, Peter Kekenes-Huskey and William A. Goddard III; ChemBioChem **8** (13): 1527-1539 (**2007**).
9. **Prediction of the 3D Structure and dynamics of Human DP G-Protein Coupled Receptor bound to an agonist and an antagonist**; Youyong Li, Fangqiang Zhu, Nagarajan Vaidehi,[1*] and William A. Goddard III' Felix Sheinerman, Stephan Reiling, Isabelle Morize, Lan Mu, Keith Harris, Ali Ardati, and Abdelazize Laoui; J. Amer. Chem. Soc. (**129** (35): 10720-10731 (**2007**)

I hereby declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Date: 2-28-08

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